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16. ABSTRACT

The first Symposium on In Situ Evaluation of Biological Hazards of Environmental Pollutants was held in Chapel Hill, NC, Dec. 5-7, 1988. The purpose of the Symposium was to discuss the application of the currently available bioassays for in situ environmental assessment and to evaluate the utility of the integrated chemical and biological data obtained under real-world condition for the assessment of human health effects from exposure to a given environment. Adverse biological effects result mostly from the complex interaction of chemical and physical components in a given environment. Such interactions that exist in the complexity of the environment often cannot be reproduced in the laboratory, but must be assessed biologically where they occur. In recent years, increased emphasis has been placed on the utilization of plants and terrestrial and aquatic animals for in situ environmental assessment. The Symposium reviewed the regulatory aspects related to industry and hazardous waste programs; the application of in situ bioassay and chemical analyses for assessing hazards to the ecological health in marine, freshwater, and terrestrial environments; and the application of in situ monitoring to human health in the work place and home. Various speakers also discussed the utility of sentinel surveillance systems and the relevancy of in situ assessment to human health and ecological integrity.

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Meeting Report

In Situ Evaluation of Biological Hazards of Environmental Pollutants

Chapel Hill, NC (U.S.A.), December 5–7, 1988

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The first Symposium on "In Situ Evaluation of Biological Hazards of Environmental Pollutants" was held in Chapel Hill, NC from 5 to 7 December 1988. The Symposium was jointly sponsored by the Office of Research and Development U.S. Environmental Protection Agency, the National Institute of Environmental Health Sciences, the National Institute of Occupational Safety and Health, the Environmental Division of the U.S. Army Construction Engineering Research Laboratory, the U.S. Army Biomedical Research and Development Laboratory, and the Proctor and Gamble Company. It was attended by over 150 representatives from industry, academia and government. The purpose of the Symposium was to discuss the application of the currently available bioassays for in situ environmental assessment and to evaluate the utility of the integrated chemical and biological data obtained under real world conditions for the assessment of human health effects from exposure to a given environment.

Adverse biological effects result mostly from the complex interaction of chemical and physical components in a given environment. Such interactions that exist in the complexity of the environment often cannot be reproduced in the laboratory, but must be assessed biologically where they occur. In recent years, increased emphasis has been placed on the utilization of plants and terrestrial and aquatic animals for in situ environmental assessment.

The Symposium reviewed the regulatory aspects related to industry and hazardous waste programs; the application of in situ bioassay and chemical analyses for assessing hazards to the ecological health in marine, freshwater, and terrestrial environments; and the application of in situ monitoring to human health in the work place and home. Various speakers also discussed the utility of sentinel surveillance systems and the relevancy of in situ assessment to human health and ecological integrity.

Opening remarks

Dr. Ann Sassman, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC (U.S.A.), provided introductory remarks on the scope of the Symposium. In her view, the most important areas to be covered were the application of the currently available bioassays for in situ environmental assessment, and the evaluation of the utility of the integrated chemical and biological data observed under real

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world conditions for the assessment of human health effects from exposure to a given environment. The concept of assessment at the origin of pollution is intriguing and important since, as stated in the announcement of this meeting, the complex interaction of chemical and physical components, from which most adverse biological effects result, cannot be reproduced — at least not easily or in all cases — in the laboratory. Thus, the concept of utilizing plants, terrestrial and aquatic animals for in situ environmental assessment is sound and offers potential for insight into both risks and mechanisms for damage to ecosystems and human health. She also described the multidisciplinary and integrated research programs at NIEHS for evaluating the impact of chemicals from hazardous waste sites on human health.

Session I — Regulatory and industrial needs in the area of biomonitoring

Dr. Barry Johnson, Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, GA (U.S.A.), discussed the role of ATSDR in concert with the U.S. Environmental Protection Agency (EPA) and the National Institute of Environmental Health Sciences in evaluating the human health effects of chemicals from uncontrolled chemical waste sites in relation to the Superfund Law. He explained that the Comprehensive Environmental Response, Compensation, and Liability Act, as amended (known as the Superfund Law), is a federal law that directs the removal of hazardous substances from the environment and evaluates their impact upon public health. The principal sources of hazardous substances are waste sites and emergency releases of toxicants. In order to address the possible human health effects of hazardous substances, the Superfund Law, as amended in 1986, authorizes a comprehensive toxicological and public health program. The overall purpose of the program is to provide toxicological information that will be of service to risk managers, health officials and the public. The Superfund Law requires ATSDR and the EPA to jointly rank those hazardous substances of greatest potential threat to health and the environment. The list of these substances now numbers 200. For each substance, ATSDR must develop a toxicological profile that describes the known toxicological properties of the substance and its human health effects. Where gaps in information exist, ATSDR and the National Toxicology Program must initiate a program of research to fill significant data needs. The data needs are to be filled by way of directed research.

Dr. Susan Norton, U.S. Environmental Protection Agency, Washington, DC (U.S.A.), in collaboration with Drs. J. Benforado and C. Zamuda of the U.S. EPA, emphasized the need for new directions for biological assessments in order to be useful in regulatory decision making. They stressed that bioassessment techniques are useful tools that can be used to evaluate the toxicity of site-specific complex mixtures using resident or laboratory-raised organisms. Although regulations based on bioassessment have been implemented under the National Pollutant Discharge Elimination Program (NPDES) program, the use of these techniques at hazardous wastes sites is at a relatively early stage of development. Most commonly, bioassessment techniques have been used to identify contaminated areas at sites, and also to measure adverse effects that have occurred at the site. In order for bioassessment to be more extensively used, advances are needed in two general areas. First, firmer links are needed between endpoints that can be measured via bioassays (e.g., death of a caged organism) to endpoints that are used in decision-making (e.g., decline of a natural population). Second, bioassessment results need to be more directly applicable to the design of treatment technologies. Specifically, new ways need to be developed to use bioassessment techniques to identify toxic agents, and if possible, establish causality. Further, the toxic agent (e.g., a specific chemical, waste fraction or medium) must be identified in a way that can be used by the engineers and chemists who are designing risk-reduction alternatives. Finally, exposure–response curves need to be developed in order to identify acceptable levels of the toxic agent. In this way, bioassessments can be used not only to document adverse effects, but also to provide input into the decisions regarding what should be remediated, and how much remediation should occur.

Dr. Janet C. Haartz, National Institute of Occupational Safety and Health, Atlanta, GA (U.S.A.), presented an overview of new directions in monitoring for occupational hazards. She pointed out that monitoring in occupational environments can be used to detect and determine, both qualitatively and quantitatively, instances of worker exposure to workplace hazards. These measurements are used to delineate exposure–disease relationships and, more importantly, to determine the remedial actions necessary to reduce workplace health risks. Efforts in occupational monitoring originally focused almost exclusively on environmental measurements of hazardous agents. More recently, there has been an increased emphasis on biochemical measurements which allow determination of the internal dose (concentration of a toxicant or its metabolite in biological specimens) or effective internal dose (biomarkers of exposure-related effects). New research directions in all three of these monitoring areas are being explored for use in NIOSH studies of exposure hazards at the work place.

Session II — Field Studies: Aquatic systems for assessing the potential hazard of the environment to ecological systems and human health

Dr. John Gentile, U.S. Environmental Protection Agency, Narragansett, RI (U.S.A.), presented results of his study on the application of a battery of in situ bioassays for assessing the impact of open-water dredged materials. In these studies, water column and benthic impacts from the disposal of 55000 m^3 of contaminated dredged material were monitored for one-year pre- and three-years' post-disposal in sentinel and indigenous organisms using a series of in situ measures representing subcellular, cellular/tissue, organismic, population and community responses. Interpretation of in situ responses was based upon environmental exposure data and laboratory-derived information on exposure–response relations, response thresholds, and reproducibility. Methods recommended for monitoring dredged material impacts include bioenergetics, somatic growth, and histopathology in *Mytilus edulis*; and somatic growth, histopathology and population growth in *Nephtys incisa*. Spatial and temporal patterns of recruitment and temporal patterns of recruitment and succession in benthic communities were measured using a rapid reconnaissance method verified with traditional analyses of community structure. Equivocal results from the sister chromatid exchange assay and the biochemical measure of energetic adenylate energy charge, indicated the need for additional methods development. The results of this and other studies suggest that measuring a group of responses provides a more reliable measure of impact. In addition, it is important to understand the relationship between sublethal responses and the survival and reproductive potential of the population when interpreting in situ responses in an ecological context.

Dr. Richard Kocan, University of Washington, Seattle, WA (U.S.A.), discussed work performed in collaboration with Dr. Marsha Landolt of the same University on the utility of herring embryos for monitoring the toxicity of marine waters. They explained that herring (Clupea sp.) are circumpolar marine species which spawn on intertidal and subtidal marine vegetation. The eggs are transparent, and sticky, and adhere to the surface of plants and other submerged objects where they incubate for 10–14 days depending on ambient temperature. They have been spawned in the laboratory and are relatively resistant to the trauma of handling, making them suitable for experimental manipulation. These characteristics have made it possible to develop an in situ method for exposing known numbers of herring eggs of known ancestry and age to polluted marine waters. Approx. 20 eggs are spawned onto glass slides, fertilized and examined after several hours' incubation to verify fertilization success. 5 slides (100 eggs) are then placed into a glass-slide holder, suspended above the sea-floor on a mooring line and allowed to incubate for a predetermined period of time. After exposure, the slides and eggs were retrieved, returned to the laboratory, examined for mortalities and allowed to complete incubation. At any time during incubation, subsamples can be fixed and examined for chromosome damage, while mortality and embyropathy are recorded up to, and following hatching. Field tests with this system have shown it capable of detecting toxicity in marine waters where natural herring embryo

mortality has been observed. The embryos have also been exposed to toxic substances in the laboratory and proved to be sensitive to a range of metals and organics.

Mr. Henry Gardner, U.S. Army Research and Development Laboratory, Frederick, MD (U.S.A.), presented data from a study conducted in collaboration with colleagues, Drs. Van der Schalie and Finch using new methods for on-site biological assessment of hazards posed by complex mixtures. In their studies, the need for biological endpoints to integrate environmental assessment was explored using teleost test animals in a mobile test facility. Acute toxicity screening and a separate carcinogenicity bioassay were conducted on the effluent from a domestic wastewater treatment plant. A computer-monitored acute toxicity technique demonstrated a rapid response to a simulated spill of 1,3,5-trinitrobenzene in river water at a concentration of 0.56 mg/l. A carcinogenicity bioassay, using a small teleost species, indicated that the effluent from this 1-million gallon per day plant was very mildly carcinogenic. This was assumed to be due to the presence of post-chlorination byproducts. Both of these new techniques for hazard assessment were found to provide useful information in these first trial experiments. The planned future use of these models in the on-site assessment of an industrial effluent and a contaminated ground water was also discussed.

Dr. Andre Jaylet, Université Paul-Sabatier, Toulouse Cedex (France), described the procedures for micronucleus tests using erythrocytes from larvae of three amphibians *Pleurodeles walt, Ambystoma mexicanum* and *Xenopus laevis*. Larvae of these species reared in water containing clastogenic substances display micronuclei in peripheral red blood cells. The results are based on a comparison of the levels of micronucleated erythrocytes on blood smears from larvae reared in water containing the clastogen with levels from control larvae reared in purified water. These tests are directly applicable to the measurement of mutagenic activity in freshwater including drinking water. These highly sensitive tests do not require prior extraction or concentration of the micropollutants. The fast response and flexibility of use make these models suitable for detection of genotoxic pollution in fresh water.

Dr. Susan Anderson, Lawrence Berkley Laboratories, Berkeley, CA (U.S.A.), discussed the results of a study performed in collaboration with Dr. Florence Harrison of the Lawrence Livermore National Laboratory, Livermore, CA, for evaluating the significance of short-term genotoxic responses in nonmammalian species that link genotoxic responses both to reproductive impairment and to the potential for detrimental population-level effects. Results of the research using the marine polychaete worm, *Neanthes arenaceodentata* were presented. These studies evaluated genotoxic, reproductive, and population-level consequences of exposure to ionizing radiation. Chromosomal aberration and sister-chromatid exchange frequencies were significantly elevated in the same dose range (0.5–5.0 Gy) in which reproductive impairment due to the induction of dominant lethal mutations was evidenced. Full lifecycle exposures also documented reduced reproductive success at a 0.55–6.5 Gy total dose when delivered over 3–4 months at a rate of 0.19 mGy/h. Decreased reproductive success occurred because of an increase in the frequency of dead embryos. There was no significant reduction in brood size or fertilization success except at the highest doses. Population-level effects were determined by age-specific increases in death rate because of the increase in embryo mortality in F_1 and F_2 broods. It was concluded that short-term genotoxicity tests can be predictive of reproductive and population-level impacts in species for which the reproductive cycle is synchronous and fecundity is relatively low.

Dr. John Arthur, U.S. Environmental Protection Agency, Duluth, MN (U.S.A.), described a study on Surface Water Toxicity Assessment in a regional watershed. He pointed out that the U.S. EPA laboratory at Duluth, MN, is developing an interactive system for analyzing water quality trends and biota impacts. This can assist in determining the applicability of regulatory strategies. Preliminary toxicity evaluations have been completed for 13 locations within the lower Fox River/Green Bay, Wisconsin watershed, a region thought to be impacted by in-place pollutants.

Study objectives were to determine where toxicity occurred and the nature of the causative agents. Samples from the water column (to within 2 inches of the bottom) were uniformly nontoxic. However, sediment-associated water (pore water) was lethal to a fish, Cladoceran, and algal species at many of the sites. Repeated sampling over time has shown sediment from the same locations to be consistently toxic. Manipulation of sediment pore water by various procedures alters toxicity. Application of further toxicity identification procedures will assist in better definition of the toxic causative agent(s) in the sediments and aid decision makers in formulating a strategy of mitigating problem sediments in this watershed.

Session III — Field Studies: terrestrial systems

Two papers presented in this session described an approach attempted by the U.S. EPA regional staff for the assessment of ecological hazards of chemical waste sites.

Dr. H. Ronald Preston, U.S. Environmental Protection Agency, Region III, Wheeling, WV (U.S.A.), emphasized the need for ecological assessment of hazardous waste sites and provided examples of work to be performed in collaboration with the U.S. Wildlife Service at two sites recommended for remedial investigation. He stressed that assessment of the environmental impact from hazardous waste sites requires the collection and evaluation of ecological data characterizing effects to the biota associated with the site. While chemical analysis is an essential first step of hazardous waste site characterization, ecological data are also needed to assess (1) the impact of the site on living resources, to allow future monitoring of cleanup effectiveness as a result of Superfund remedial actions, and (2) to meet the information needs of responsible natural resource agencies (which are required under Comprehensive Environmental Response Compensation Liability Act (CECLA) to evaluate the impacts of NPL sites on the "trust" natural resources of the agencies). In order to address the need for ecological evaluations at Superfund sites in Region III, representatives from the U.S. EPA and Federal natural resource agencies have formed a "Bioassessment Work Group" that meets monthly to provide technical recommendations to Superfund project managers on biological studies that may be needed at specific sites. Examples of site investigations recommended by the Region III bioassessment work group were provided for the Chisman Creek (Virginia) site and the Wildcat (Delaware) site and were discussed in this presentation. The U.S. Fish and Wildlife Service conducted a remedial investigation at the request of EPA at the Chisman Creek Superfund Site to assess the environmental impacts of hazardous substances at the site. The site consists of abandoned sand and gravel borrow pits that were filled with flyash from an electric power generating station. A biological assessment was conducted to (1) determine the concentrations of metal contaminants in the aquatic environment, (2) assess the toxicity that surface waters and sediments pose, and (3) generate information necessary to determine remedial actions to eliminate or mitigate the impacts of contaminants at the site. Results indicate that the surface water, sediment, fish and aquatic vegetation at the site contain elevated levels of several metal contaminants. Toxicity tests showed toxic conditions in one of the freshwater ponds and Chisman Creek sediments. Histopathological studies and a benthic survey did not indicate any significant biological impacts.

The Wildcat Landfill Site in Delaware was reviewed by the bioassessment work group and a series of ecological assessments was recommended. The U.S. Fish and Wildlife Service conducted portions of these assessments for the remedial investigation. The site is a former estuarine wetland partially destroyed by a landfill containing hazardous waste. The biological assessment was designed to (1) delineate the wetland, (2) determine the extent of contamination and toxicity in surface water and sediments of the onsite pond, wetlands, and adjacent St. John's River, and (3) determine potential impacts and bioaccumulation pathways in the vegetative and wildlife communities. Results to date, including those of population and histopathological studies, indicate no abnormalities in the terrestrial community. Results for aquatic communities are not yet available. Information may be used to develop mitiga-

tive measures for the wetlands during remediation at the site.

Dr. Alice Fritz, National Oceanographic Atmospheric Administrations (NOAA), Philadelphia, PA (U.S.A.), discussed the utilities for bioassessment for remedial processes and pointed out that bioassessment can be particularly useful as a tool in (1) site screening for Preliminary Assessment, (2) characterizing the extent of contamination and potential impact to natural resources in Remedial Investigation, (3) establishing clean-up levels, (4) impact evaluation and prediction of risk in Endangerment Assessment, and finally, (5) monitoring and evaluation of success of the remedial action. The possible role and the limitation of different bioassessment approaches and methodologies during these different stages in the hazardous waste site remedial process were discussed in this presentation. It is important to note that bioassessment is a continually changing and developing field. In situ methodologies, as they are accepted, can make an important contribution to bioassessment and ecological evaluations used in the Superfund remedial process.

Dr. R.S. Athwal, New Jersey College of Medicine and Dentistry, Newark, NJ (U.S.A.), presented preliminary data on the development of an assay using kinetochore staining in bone-marrow micronucleated erythrocytes for detecting induced aneuploidy and clastogenicity. The detection of micronuclei formation in young erythrocytes is a commonly used in vivo test to determine the genotoxic potential of environmental agents. Abundance of scorable polychromatic erythrocytes and easy identification of micronucleated erythrocytes make this assay particularly suitable to assess the induced cytogenetic damage. Micronuclei formation results from the failure of a chromosome to incorporate into the main nucleus at anaphase either due to the lack of a centromere or spindle disfunction. As such, micronuclei could originate from acentric chromosomal fragments resulting from clastogenic effects as well as from intact chromosomes due to interference with movement of a chromosome to the poles. Micronuclei in polychromatic erythrocytes cannot be classified for the presence of acentric chromosome fragments or an intact chromosome by standard Giemsa staining, but kinetochore proteins are recognized by CREST antibodies, making it possible to differentiate populations of micronuclei with and without kinetochores. Preliminary validation of this bioassay was performed with vinblastine and X-rays. This bioassay has potential for application to in vivo hazard identification and for further characterization of active and abandoned chemical waste sites.

Dr. John W. Bickman, Texas A and M University, College Station, TX (U.S.A.), discussed the use of flow cytometry to monitor and assess the effects of environmental mutagens on wildlife populations. He demonstrated that flow cytometry is a rapid and accurate means of measuring DNA content variation within and among cell populations. Specific mutagens have been shown to cause mutation in bacteria, chromosomal aberrations in eukaryotes, increased variation in DNA content, cancer, and heritable genetic defects in mammals. Flow cytometry is particularly well suited for the demonstration of clastogenic activity because large numbers of cells from virtually any tissue can be assayed, and the procedure is readily applicable to virtually any organism. Wildlife populations exposed to environmental mutagens, including petrochemicals and low level ionizing radiation, show increased variation in the DNA content of G_1 cell populations as well as evidence of mosaicism. The efficacy of the procedure was demonstrated on natural populations of terrestrial rodents and aquatic turtles occupying well-characterized hazardous waste disposal sites in Texas and the Savannah River Plant in South Carolina.

Dr. Edward Novak, United States Army, Construction Engineering Research Laboratory, Champaign, IL (U.S.A.), discussed a comprehensive, systematic approach to ecosystem analysis based on identification and quantification of factors which define the condition or state of an ecosystem in terms of health criteria. The classical definition of an ecosystem couples interacting living organisms and nonliving components of the environment to form one physical system (Tansley, 1935) and grew from the recognition that definable and describable units existed in nature.

Ecosystem analysis has been advanced by an improved understanding of how ecosystems are structured and how they function. Ecology has advanced from an emphasis on natural history to consideration of energetics, the relationships and connections between species, hierarchies, and systems theory. Still, we consider ecosystems as entities with a distinctive character and individual characteristics. Measures of human or nonhuman animal health, and the clinical analysis of factors which contribute to a definition of a state of health, provide useful analogs to the problems faced by environmental managers attempting to maintain the integrity of ecosystems (Schaeffer et al., 1988). As found in human/nonhuman animal health studies, disease states of ecosystems must be recognized before disease is of clinical magnitude.

Dr. Lloyd Fitzpatrick, University of North Texas, Denton, TX (U.S.A.), described his studies for the development of earthworm immunoassays for evaluating biological effects of exposure to hazardous materials. In these investigations, the humoral cell-mediated, and phagocytic immune functions in earthworms, *Eisenia foetida* and *Lumbricus terrestris*, are evaluated for use in assessing immunotoxic potential of environmental pollutants to mammals. Experiments on the ability of *L. terrestris* leukocytes to form secretory (S) rosettes and phagocytize erythrocytes were discussed in relation to environmental exposure concentration, tissue compartmentalization, and uptake/depuration dynamics of Aroclor 1254. Using 5-day filter paper contact exposure, LC_{50} and LD_{50} were 0.30 μg/sq. cm and 1139.63 μg/g whole-worm dry weight. Nominal filter paper concentration of 0.005 μg/cm and 76.5 μg/g whole-worm dry weight concentrations were sufficient to suppress both S-rosette formation and phagocytosis. Suppression and return of immune functions correlated with carcass and coelomic tissue concentrations during uptake and depuration.

Session IV — Sentinel surveillance systems

Dr. Larry Glickman, Purdue University, W. Lafayette, IN (U.S.A.), discussed the use of pet animals as sentinels of environmental health hazards. He explained that the word sentinel is derived from the Latin word meaning "to avoid danger by any means" and that since the canary was first used to alert miners to the presence of hazardous gases, there has been interest in the concept of animals as sentinels. Epidemiologic methods applied to animal sentinels can generate data for use in risk assessment. Such studies can: (1) confirm that natural exposures to xenobiotics which cause cancer in humans cause the same cancer in animals, but with a shorter latency, (2) identify xenobiotics that could potentially cause cancer in humans before they would otherwise be detected, and (3) clarify the relationship between a suspected environmental hazard for humans and various disease outcomes. These attributes of an animal sentinel were illustrated with studies in pet dogs of mesothelioma and asbestos, bladder cancer and insecticides, and breast cancer and diet.

Dr. Stephanie Ostrowski, Center for Disease Control, Atlanta, GA (U.S.A.), reviewed the reports published in the literature on the utility of animal models for investigating the pathophysiology of lead poisoning over the last two decades. She stressed that, in the future, animals may prove to be of even greater value as sentinels for the presence of lead hazards in communities. Childhood lead poisoning prevention programs need to incorporate new and more sensitive methodologies to determine target communities for intervention, because they will be concerned with lower concentrations of blood lead (PbB) than in the past. Surveillance of PbB in community adults is not sufficiently sensitive to achieve this and surveillance of children (the population of interest) is costly, time-consuming, and may have poor compliance rates in areas where no perceived lead hazard exists. Results of recent pilot and field studies indicate the PbB surveillance of dogs in neighborhoods of interest may be accomplished rapidly and at minimal cost. In several communities studied, mean PbB results appear to have value in predicting mean PbB values for children in the same community; dogs appear to have mean PbB values approximately twice that of children.

Dr. John M. Grizzle, Auburn University, Auburn, AL (U.S.A.), described the high prevalence of neoplasms in several fish populations in North America. Locations of these fish include the: Hudson River estuary; Fox River near Chicago; Western Lake Ontario; Black River near Cleveland, OH; Buffalo, New York, area; various regions of Puget Sound, Washington; Chesapeake Bay; Boston Harbor; Torch Lake, Michigan; and a pond in Alabama that received chlorinated sewage effluent. The most commonly affected species of fish are demersal (bottom-dwelling), suggesting that contact with contaminated sediments or consumption of benthic organisms is related to the occurrence of neoplasm. However, some nondemersal species have been reported to have a high prevalence of neoplasms. Neoplasms found in fish from polluted waters are usually in the liver (hepatocellular carcinoma and cholangioma) or skin (papilloma). Many of the polluted locations have complex mixtures of chemicals so that it is difficult to determine which chemical(s) triggered the neoplasms. The reports reviewed vary in thoroughness, but generally support the conclusion that certain types of neoplasms in fish can be caused by the environmental conditions existing in waters polluted with chemical carcinogens.

Dr. John McCarthy, Oak Ridge National Laboratory, Oak Ridge, TN (U.S.A.), described a research approach to develop and validate the concept of using wild animals and introduced caged animals near hazardous waste sites as (1) sentinels of bioavailable contaminants, (2) predictors of adverse ecological effects, and (3) surrogates to estimate the potential exposure and risks to humans living near these sites. Evidence of exposure in animals on the site provides a temporally-integrated measure of bioavailable contaminant levels and is therefore much more relevant to the potential risk to humans than is the analytically measurable concentration of contaminants in the soil, water, or air. The research approach monitors biomarkers (biochemical or molecular indicators of exposure) and measures body burden of persistent compounds (such as PCBs) in wild animals captured on a hazardous waste disposal site and in adjacent uncontaminated areas to identify and quantify the potential for exposure to bioavailable contaminants. Unexposed animals confined at sites confirm the potential for environmental exposure. Relationships between biomarker response and adverse ecological effects are determined from measures of animal health and population structure. The potential risk to humans is extrapolated from the animal exposure data using pharmacodynamic models.

Dr. Bruce P. Dunn, British Columbia Cancer Research Center, Vancouver (Canada), discussed the use of DNA–carcinogen adducts in fish as a tool for measuring the effective biological dose of carcinogens. Brown bullheads from Great Lakes areas contaminated with polycylic aromatic hydrocarbons exhibit an elevated rate of liver cancer. Using ^{32}P-postlabeling techniques, DNA adducts of aromatic environmental carcinogens were measured in livers of brown bullheads from a number of sites with different tumor rates. Chromatograms derived from fish from areas contaminated with polycyclic aromatic hydrocarbons exhibited a broad diagonal radioactive zone which is interpreted as resulting from multiple overlapping adduct spots. In a limited survey, DNA adduct levels in fish appeared to parallel the tumor rates in wild fish populations. Adduct levels in fish from a given area were less variable than the levels of fluorescent aromatic hydrocarbon metabolites measured in the bile of individual fish. This suggests that when few fish are available, adduct measurement would be more suitable than chemical analysis of bile levels of carcinogen metabolites for assessing the uptake of carcinogens by fish. The measurement of DNA adducts appears to have considerable potential for directly measuring genetic damage in wild animal populations caused by environmental carcinogens.

Dr. Te-Hsiu Ma, Western Illinois University, Macomb, IL (U.S.A.), presented work in which in situ monitoring of environmental clastogens was performed using the Tradescantia–Micronucleus bioassay (Trad–MCN). He has shown that the Trad–MCN is a versatile eukaryotic test, which is capable of detecting very low concentration of clastogens in the air or in water directly at the site of pollution. The gaseous agents can be diffused easily into the flower buds and the liquid agents

can be absorbed readily into the target cells which are undergoing meiosis. The extra high sensitivity of this test is due to the fact that the clastogenic action can be focused on the period of crossing-over, a process involving breakage-fusion of chromatids. Micronuclei formation resulting from the breakage of chromatids can be attained not only by the excision of the DNA molecules, but also the interference of DNA repair in the process of fusion.

The monitoring procedure includes: exposing the plant cuttings (15 per site) at the site of pollution (3–12 h), allowing a 24-h recovery time, fixing inflorescences, preparing slides, and scoring micronucleus frequencies. Generally, a field control and a laboratory control were included with the treated groups. The monitoring work was usually repeated 3 times to ascertain the results. Current results of the in situ monitoring of air pollutants were obtained from radon-contaminated farm houses, trailers, cigarette smoking rooms, newly-painted offices, and storage rooms with volatile chemicals. Positive results were obtained at all sites as compared to the controls. Positive results were also obtained from the in situ monitoring of water pollutants from a local reservoir and a polluted creek using a floating device, the "Aquatoon", to carry the plant cuttings.

Dr. Lawrence Harshman, University of California, Davis, CA (U.S.A.), described the potential utility of *Drosophila melanogaster* as a model for in situ biomonitoring. It is generally known that *Drosophila melanogaster* is genetically well-characterized and large numbers can be reared inexpensively. These attributes make it useful as an indicator species for environmental contaminants. The flies can be reared in situ to monitor the effects of volatile toxicants. Potentially contaminated soil or water samples can be tested by exposing larvae or adults in the laboratory. Cytotoxic effects can be assessed at different life stages and a variety of mutation tests are available. Induction of detoxication enzyme activity may be relatively specific and a sensitive indicator of environmental hazards. Molecular genetic manipulations can be used to enhance the utility of tests to monitor human health hazards.

Session V — Integration of data for effective problem solving and assessment

Dr. Tabor M. Wilson, University of Cincinnati Medical Center, OH (U.S.A.), described state-of-the-art methods for the detection of chemicals at very low concentrations in the environment and the possibility of interference by artifacts. He stressed that due to the presence of both known and unknown sites and sources of environmental pollutants in many industrialized regions, there exists the possibility for release of hazardous anthropogenic organics into the environment that present a potential hazard to human health. Many of these releases remain undetected until after the fact; i.e. endpoints of exposure surfaces in the indigenous population, chemical monitoring/biological monitoring, or survey programs show contamination, threat of release or that the pollutants at the site of the contamination or release affect the indigenous flora or fauna. An approach to early detection of environmental pollutants, or release of these pollutants, is the use of in situ monitoring methodologies via sentinel organisms. These situations require an approach by the environmental analytical chemist that provides data appropriate to assess and evaluate the situation so that effective measures can be instituted. The acquisition of such data requires analyses at trace levels, i.e. ppb or lower, thereby increasing the probability of interference from (1) artifacts of isolation or (2) inadequately resolved components in the complex mixture under analysis. Considering these problems, an overall analytical strategy to such cases of assessment and evaluation of environmental pollutants was discussed.

Dr. W.J. Berge, University of Kentucky, Lexington, KY (U.S.A.), described the utility of in situ toxicological monitoring for quantifying the ecological effects of hazardous wastes. In developing test systems for evaluating chronic effects of municipal and industrial point-source discharges, samples of effluents were tested simultaneously in the field and the laboratory. Though optimal sample preservation and minimal storage time (i.e. 12–24 h) were observed, laboratory-tested samples produced substantially less biological activity as measured by embryopathic effects on fish and

amphibians (e.g. embryonic mortality, delayed hatch, teratogenesis, growth inhibition). Certain waste samples, which were toxic in the field, produced no effect in the laboratory, clearly indicating the prospect for "false negative" results in laboratory screening. These studies were conducted under different field conditions, using static and continuous exposures, and involved a wide variety of toxicant mixtures. Results support the need for, and applicability of, in situ toxicity testing to evaluate properly the environmental health effects of hazardous substances. In subsequent investigations, biomonitoring studies were conducted on three point-source, impacted streams. Results of conventional laboratory toxicity tests, in situ toxicity tests, instream chemical measurements, and ecological endpoints were analyzed and compared for sensitivity and reliability for measuring or predicting ecological effects of hazardous wastes. In each study, 6–8 monitoring stations, ranging from high to low impact, and upstream control stations were studied. On-site "short-chronic" toxicity test with fish embryos and larvae produced results which correlated closely with independent ecological parameters (e.g., fish diversity; species richness, diversity, density, dominance of benthic invertebrates). Species richness and diversity generally were the most reliable ecological endpoints. Results of laboratory toxicity tests were useful but less precise than in situ tests for predicting ecological effects. On-site toxicity tests of effluent serial dilutions produced dose–response data that correlated closely with degrees of ecological impact observed for downstream sequences of monitoring stations. Though test designs may vary, the principle of in situ toxicological monitoring is also applicable to terrestrial ecosystems. Conclusions from studies to date support the feasibility of a broad-based in situ monitoring program for evaluating ecological and health effects of hazardous substances.

Dr. Beverly Weeks, The College of William and Mary, Gloucester Point, VA (U.S.A.), presented an integrated chemical, pathological and immunological study to assess environmental contamination of the Elizabeth River. This river, an estuarine tributary of the James River in Virginia, is highly contaminated by agricultural, domestic and industrial pollutants. Polynuclear aromatic hydrocarbons (PAHs) have accumulated in the sediments to dangerous levels. Chemical analyses have shown a gradient of PAH concentrations increasing in an upstream direction. At the most contaminated site, benzo[*a*]pyrene (BaP) concentrations in the top 2 cm approached 10 μg/g, while at a depth of 30 cm BaP concentrations exceeded 200 μg/g. Total PAH concentrations of 13 000 μg/g at the 30-cm depth have been recorded. Finfish from the Elizabeth River exhibited high incidences of hyperemia, fin erosion, ulcerations and lens cataracts while those from the nearby Nansemond reference estuary did not. Internal lesions included: hypertrophy, hyperplasia, and growth deformities of gill tissues; neoplasms of the buccal and gill cavities; and inflammatory, necrotic, preneoplastic foci and macrophage aggregates in hepatic or pancreatic tissue. Several eye lesions, including a tumor of the lens epithelium, retinal deformities, and hypertrophy and hyperplasia of the choroid gland, were also seen. Fish exposed to contaminated Elizabeth River sediments in the laboratory began to die within 7–8 days while survivors exhibited cataracts, ulcerations, fin erosion, gill lesions, liver and pancreatic alterations, and lowered hematocrits and body weights.

The cellular immune system was found to be compromised in fish captured in the Elizabeth River and in those exposed to Elizabeth River sediments in the laboratory. Macrophages from fish exposed to toxic contaminants showed responses which were suppressed or elevated depending on the species and activity tested. Results of gross and histopathological studies and immunological assay techniques were presented with the aim of providing reliable bioindicators of the effects of environmental contamination on fish health.

Dr. Martyn Smith, University of California, Berkeley, CA (U.S.A.), discussed the utility and limitations of biomarkers of organic chemical exposure for hazard assessment. It was pointed out that there are a large number of potential routes for human exposure to chemicals at hazardous waste sites. This makes quantitation of the exposure difficult and requires new approaches to

human dosimetry. Organic chemicals are usually metabolized in the body to reactive intermediates which bind to macromolecules, such as DNA, and form adducts. These adducts could therefore serve as biomarkers of exposure. Various methods for characterizing and quantitating DNA adducts have been developed and applied to occupationally exposed human populations. Unfortunately, these methods are not ready for routine use. Moreover, rates of repair and life-times of most DNA adducts in human cells are not known. Adduct measurement, whether to DNA or protein, offers the advantage of chemical identification, but suffers from the fact that its relationship to toxic response is unclear. Various indices of genetic damage have therefore been developed, but these suffer from the fact that the chemical agent responsible is usually not identifiable. They do, however, offer the advantage of being at least one measure of toxic response. These indices include markers of chromosomal damage, such as SCEs and micronuclei, as well as mutational assays. While these methods are suitable for workplace monitoring, none are presently sufficiently sensitive, selective, or quantitative to allow precise measurement of low level, mixed chemical exposure. Much research is therefore needed before we can adequately quantitate and prevent chemical exposure and potential adverse effects to populations living near uncontrolled hazardous waste sites.

Dr. Ronald J. Kendall, Western Washington University, Bellingham, WA (U.S.A.), described the use of wildlife for on-site evaluation of bioavailability and ecotoxicity of toxic substances found in hazardous waste sites. Wildlife species which live in and around hazardous waste sites provide an opportunity for in situ evaluation of bioavailability and ecotoxicity of toxic substances. This approach integrates environmental chemistry, food resource sampling, habitat use assessment, and wildlife species occurrence. Exotoxicological, biomedical, immunotoxicological and reproductive endpoints can be chosen based on associated site contamination. Current research involves enhancing European starling populations on-site using artificial nest boxes. Reproductive success and foraging behavior are monitored through observation and radiotelemetry. Telemetry bioavailability and contaminant transfer are verified through invertebrate food item analysis.

Wildlife collected on site are used to determine exposure to toxic compounds and to assess biomedical and immunotoxicological endpoints (e.g., cytochrome P450 activity). The use of wildlife in assessing hazardous waste sites provides an effective method to determine ecotoxicity and an early warning system for potential human health impact. Data can be used to rank sites and to evaluate remedial action based on toxicity measured in situ.

Dr. Thomas Murphy, U.S. Environmental Protection Agency, Corvallis, OR (U.S.A.), presented the capabilities and limitation of approaches to in situ ecological evaluations. He advised that ecological assessment is in a state of rapid evolution and growth, both in its concepts and in its techniques. The more traditional approaches, toxicological analysis and field survey, have significant limitations for current demands. Widely variable ecological and exposure conditions preclude a few generally applicable assessment schemes. Each situation requires individual design, however, some generalizations are possible. Two considerations are often given insufficient attention for a useful and scientifically sound assessment. One is the clear definition of an ecological endpoint for assessment that: (a) is meaningful for policy, regulatory, or management decisions, and (b) is itself measurable or capable of being estimated from measurable parameters. The second is the selection of an appropriate assessment approach. Usually this involves integration of both toxicological and field ecological information. Selection of endpoints and approaches were illustrated through examples.

Dr. Marja Sorsa, Institute of Occupational Health, Helsinki (Finland), discussed the use of human biomonitoring in the identification of environmental genotoxic hazards. She reasoned that the rapidly growing number and concern of environmental contaminants and the complexity of the exposures, both quantitatively and qualitatively, gives the key motivation for the development of methods applicable to in situ monitoring. A further objective is the use of suitable biological

indicator organisms, other than humans, to serve as an early warning signal for potential human health hazards. In special occasions, groups of humans exposed to an environmental contaminant occupationally may take the role of a biological indicator species in an in situ exposures situation. The work related exposures are frequently more exactly characterized and the exposure levels are considerably higher than in the general environment. Such situations may serve as a model case in developing suitable exposure assessment methodologies for the public and the general environment. Three such model cases were discussed in the presentation. Of these, styrene and ethylene oxide comprise single compound entities, while monitoring of exposures in the rubber industry gives an example of a complex multiexposure environment.

Dr. Marvin Legator, The University of Texas Medical Branch, Galveston, TX (U.S.A.), presented the problems in evaluating the mutagenic effects of complex chemical mixtures. He pointed out that there is no approach to the multifaceted mixture problem that is free of significant problems. A careful evaluation must be made of the various alternatives, and a special effort must be made to avoid the errors of the past, such as an attempt to rely on data from non-mammalian studies. Two areas that should be pursued include the use of in situ animal models and the utilization of a multi-endpoint comprehensive laboratory animal study. The in situ animal monitoring study can be divided into several categories including: (a) the use of domestic and farm animals, (b) the evaluation of feral animals, and (c) the deliberate placement of animals in areas adjacent to a potential source of pollution. The laboratory studies can be modeled after specific polluted areas, such as waste disposal sites. The ratio of the major pollutants in these areas should be formulated for conduction of multi-endpoint rodent studies. If the sites studied contain pollutants representative of many areas throughout the country, the results generated from in situ monitoring or laboratory studies may be indicative of health hazards in several locations throughout the country. Toxicological information could also serve as the driving force for epidemiological studies indicating the most likely endpoints to exploit in human studies.

Dr. P.J. Peterson, Monitoring and Assessment Research Centre, London (Great Britain), discussed the chemical aetiology of endemic disease — a global perspective. In this discussion, he stressed that the human health effects caused by excessive exposure to elements such as arsenic, cadmium, lead, and mercury are well-documented for both occupational workers and for specific population groups living in the vicinity of the pollutants, whether from natural geochemical anomalies or industrial discharges. The extent of other health effects from the so-called endemic diseases, fluorosis, iodine-deficient goitre, selenosis, Keshan Disease and Kaschin-Beck Disease are less well-understood and the chemical aetiology not at all clear for the latter two diseases. Although the title "endemic diseases" would imply that their occurrence is restricted to a country, this is not the case. Several of these diseases are global in nature, or at least of regional significance. Estimates of the numbers suffering from such diseases approach one billion people.

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